

ScreenTape® P200 accurately analyses Maltose-Binding Protein tagged (MBP-Tag) proteins after purification on amylose columns

Tape Used – ScreenTape P200

Time to result – Less than one minute per sample

Sample volume per test – 2µl

Specifications

Sizing range: 10 to 200kDa

Molecular weight accuracy (average deviation) <10%

Molecular weight precision ≤2% CV

Suggested sample conc.: 100 to 1000µg/ml

Typical sensitivity: 5µg/ml

Quantitative range: 5 to 5000µg/ml

Analysis Results

Automated peak location and annotation

Automated protein sizing and % purity value

GLP standard report and print-out

ScreenTape P200 is a fully automated electrophoresis analysis method for proteins of 10 to 200kDa. ScreenTape P200 simplifies the screening of protein fractions from MBP-Tag purifications because of its ease of use and integrated analysis. P200 is fully compatible with the MBP-Tag buffer system allowing you to load samples directly onto the platform for rapid analysis. This allows you to streamline your protein purification workflow, by shortening the time to result on intermediate protein QC checks.

Introduction

MBP is commonly used to tag recombinant proteins because of its high affinity for amylose resin, which makes it possible to achieve protein purification in a single step.

MBP-Tags can be placed at the N- or C- terminus and are most useful when the target protein is difficult to solubilise, as they tend to improve its yield recovery. Fractions collected during the purification process are routinely tested to monitor protein expression levels and molecular weights using an electrophoresis method. ScreenTape P200 demonstrates both accurate and reproducible performance for protein fraction analysis and is a method of choice for this procedure. Purified MBP-Tagged proteins are accurately sized, whilst impurities are precisely detected, sized and quantified.

Materials and Methods

A 50µl sample containing 2mg/ml MBP fusion protein of 89kDa (DSTT, University of Dundee) and two contaminants: 0.1mg/ml β-galactosidase (116kDa) and 0.05mg/ml Lysozyme (14kDa) (Sigma), in wash buffer (20mM Tris pH7.4, 200mM NaCl, 1mM EDTA, 1mM DTT) was added to a Zeba micro-spin column (Pierce) containing 25µl prepared amylose sepharose (NEB) and incubated on ice for 30 minutes. Following affinity binding, the mobile phase was collected in a single fraction by centrifugation to recover the unbound proteins. Removal of contaminating proteins was carried out by washing the column seven times by centrifugation after addition of a column volume of wash buffer, generating seven fractions. Three 25µl volumes of elution buffer (20mM Tris pH7.4, 200mM NaCl, 1mM EDTA, 1mM DTT, 1mM maltose) were used to elute the MBP fusion protein from the amylose column. Aliquots of 2µl were collected from the contaminated protein material, wash 1, elution 1 and 2 and analysed on ScreenTape P200.

ScreenTape P200 Analysis Procedure

All samples were pre-stained and prepared for analysis on ScreenTape P200 according to the Lab901 protocol, and transferred to the TapeStation® with ScreenTape P200 and tips for sample loading. Pre-staining of samples during the sample preparation stage avoids lengthy staining and destaining procedures that are common to SDS-PAGE methods. After clicking “START” on the software driven menu, full analysis of the samples was achieved and archived, with no user intervention.

Results

Lab901-GeneTools™ software presented analysed protein results in under one minute per sample (displayed as a screen-grab in Figure 1), therefore allowing fast and accurate monitoring of MBP-Tag purification products. These results contain information on the protein molecular weights and peak volumes, which were automatically calculated and presented in a gel image, electropherogram and table. Results in Figure 1 also show that each lane contains in-lane markers (red) that allow reproducible

protein sizing. Peaks in all lanes were automatically annotated and could also easily be compared using the electropherogram overlay function of the Lab901-GeneTools software (as shown in the profile overlay in Figure 2), making it simple to see differences in levels and types of impurities. With these results we demonstrate that ScreenTape P200 can accurately separate and size MBP-Tagged proteins in fractions collected from amylose affinity purifications, which frequently contain salt concentrations of up to 200mM and additives such as maltose, DTT and EDTA. This reagent tolerance avoids any buffer exchange step during sample preparation, which is often required with other microscale automated electrophoresis systems. The data was automatically archived and could be printed to a GLP compliant report with a single mouse click.

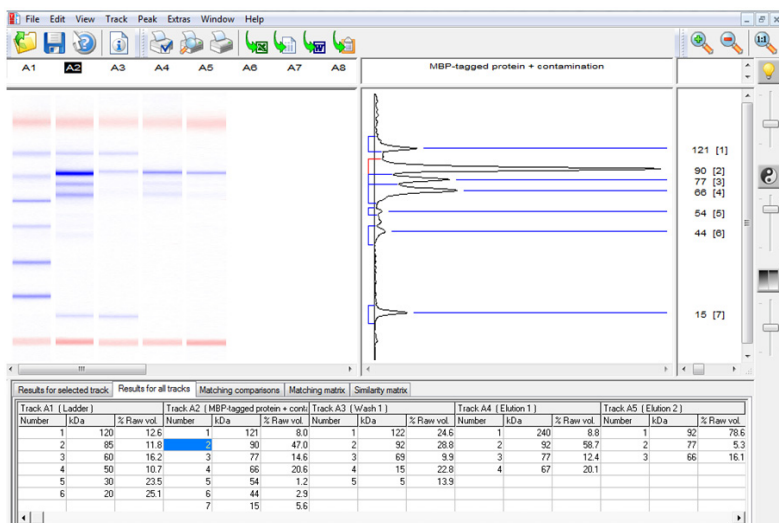


Figure 1: Analysis of amylose affinity gel fractions by the ScreenTape P200 system – Lane 1 contains the P200 molecular weight standard with proteins at 120kDa, 85kDa, 60kDa, 50kDa, 30kDa and 20kDa. Lane 2, also shown in the electropherogram, contains the MBP-Tagged protein with β -galactosidase and lysozyme contaminants, as well unidentified contaminants at 77 and 66kDa. Lane 3 contains wash 1, Lanes 4 and 5 contain elution fractions 1 and 2 respectively, which contain the target protein.

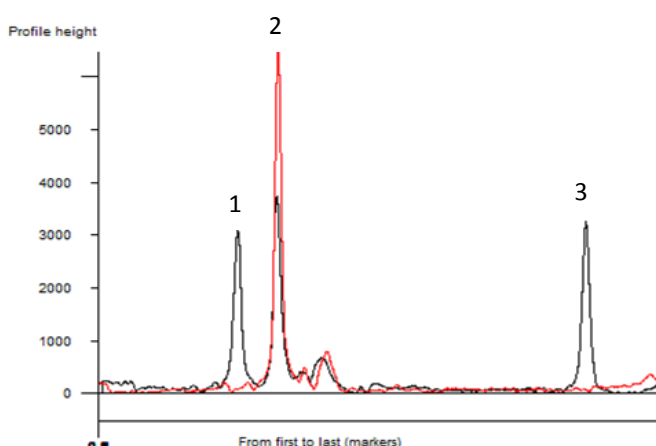


Figure 2: ScreenTape P200 can display overlaid protein profiles, making fraction comparison easy. Here we show the overlay of electropherograms from wash 1 in lane 3 (black) and the purified protein from lane 5 (red). Peak 1: β -galactosidase (sized at 121kDa), Peak 2: MBP-fusion protein (sized at 89kDa), Peak 3: Lysozyme (sized at 15kDa).

Benefits of using ScreenTape P200 for monitoring the purification of MBP-Tagged proteins

- **A more efficient workflow** - The P200 platform streamlines protein fraction QC checks during your MBP-Tag affinity purification workflow. At less than one minute per sample, ScreenTape P200 is efficient and eliminates the protein electrophoresis bottleneck, allowing you to focus on the next stage of your experiment.
- **Safe** - P200 reagents are pre-packaged and self-contained avoiding messy manual interventions or exposure to potentially harmful reagents.
- **Improved reproducibility and accuracy** - Automation and pre-packaged reagents mean ScreenTape is accurate and reproducible. Results from several different analyses or batches can be precisely compared using the electropherogram overlay function.
- **GLP compliant** - Lab901's integrated GeneTools software makes data analysis, archiving and retrieval simple. You can produce GLP compliant reports with one mouse click.
- **Cost effective** - Unused lanes on ScreenTape can be used at a later date thanks to a barcode that is unique to every tape. There is no need for samples to be batched to fill every position on a piece of ScreenTape.

For a full list of application notes covering additional protein purification analysis methods, please visit www.lab901.com.

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